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Physical Models of Living Systems 2nd ed, new chapter: Random Walks on an Energy Landscape

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Abstract

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Disciplines

Biological and Chemical Physics | Physical Sciences and Mathematics | Physics | Statistical, Nonlinear, and Soft Matter Physics

Comments

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CHAPTER 6

Random Walks on an Energy Landscape

[The] phenomena of Nature resemble the scattered leaves of the Sibylline prophecies; a word only, or a single syllable, is written on each leaf; but when every fragment is replaced in its appropriate connection, the whole begins at once to speak a harmonious language.

—Thomas Young, 1807

6.1 SIGNPOST: *FIRST PASSAGE*

First-year physics generally begins with a semester about mechanics, which doesn't feel particularly "life-like." But mechanics becomes much more relevant to cell and molecular biology when we acknowledge the incessant thermal motion that dominates the nanoworld.

One thread that runs throughout molecular cell biology is that:

- Some pairs of molecules bind, whereas other pairs ignore each other.
- Binding can be affected by mechanical forces—the same forces studied in first-year physics—and this effect (**mechanochemistry**) can enable cells to sense their environment (**mechanobiology**).

This chapter will begin to explore some of the strands in that thread, including some surprising applications to immunology.
The Focus Question is
Biological question: How can pulling two things apart strengthen their bond?
Physical idea: Bond breaking is a *first passage* process, controlled by the lowest energy barrier, and this can increase upon moderate loading.

6.2 ONE PARTICLE

6.2.1 The free random walk is a model for molecular diffusion

Ultimately, we will study the specific interaction of one molecule with another. A biological example could be a signaling molecule that can bind to a receptor on a cell surface; depending on context, such a molecule may be called a "ligand," "antibody," or "agonist" for the receptor if it specifically matches a binding site on the latter. Before we turn to such situations, however, let us start with the motion of just one molecule of interest, suspended in a milieu of other molecules that it *doesn't* bind.

Problem 3.4 explored the idea that free diffusive motion of such a molecule, or of a larger suspended particle such as a pollen grain in water, could be modeled as a random walk. That is, we choose a short time interval Δt and suppose that the particle of interest suffers a small kick from thermal motion of its surroundings every Δt. We

assume that each kick moves the particle a distance Δx in a randomly chosen direction. This chapter will simplify by considering situations in which only one direction of motion interests us; hence, a "randomly chosen direction" means a choice directed to the right or the left.[1]

The time interval Δt could literally be the time between successive molecular collisions, but it could instead be a longer time if we choose a suitable Δx to summarize the effects of a string of many kicks. Whatever choices we make, you found in Problems 3.4 and 4.6 that this simple model does accurately predict the observed distribution of final particle locations after a total elapsed time $t = N\Delta t$ that corresponds to many steps. In particular, it correctly predicts that the distribution is Gaussian, with variance that increases linearly with elapsed time.[2]

Some illustrative values are $\Delta x = 1\,\mu\mathsf{m}$ and $\Delta t = 1\,\mathsf{ms}$; then the resulting random walk resembles the motion of a micrometer-size bead in water. It's useful to abbreviate by defining the **diffusion constant** as

$$D = \Delta x^2/(2\Delta t). \tag{6.1}$$

Thus $D = \frac{1}{2} \times 10^{-9}\mathsf{m}^2\mathsf{s}^{-1}$ in the illustrative case just mentioned.

6.2.2 The random walk with drift is a model for driven motion

Next, suppose that our particle is subjected to a constant external force. For example, if its density is different from that of the surrounding fluid then it will feel a net pull from gravity.[3] We then expect that a systematic migration along the direction of the applied force will be superimposed on the random Brownian motion. The systematic motion, or **drift velocity**, is observed experimentally to be simply proportional to the force f, with a constant of proportionality called the particle's **mobility**. Equivalently, we can define a **viscous friction coefficient** ζ as the inverse of mobility:

$$\langle v_{\text{drift}} \rangle = f/\zeta. \tag{6.2}$$

Thus, ζ carries units such as $\mathsf{kg}\,\mathsf{s}^{-1}$.

Our physical model for diffusion can accommodate drift: We simply take the probabilities of left and right stepping to be unequal. By working out the expectation of net displacement after many steps, we find that $\langle v_{\text{drift}} \rangle = (\mathcal{P}_+ - \mathcal{P}_-)\Delta x/\Delta t$. Moreover, some forces, including gravity, can be written as minus the derivative of a potential energy function U.

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[1] T2 In a bound complex, "right" and "left" may refer to directions along the reaction coordinate describing the lowest-energy pathway to dissociation.

[2] You saw this in Problem 5.11.

[3] More biophysical examples include motion under the artificial gravity in a centrifuge (**sedimentation**), or in an applied electric field (**electrophoresis**).

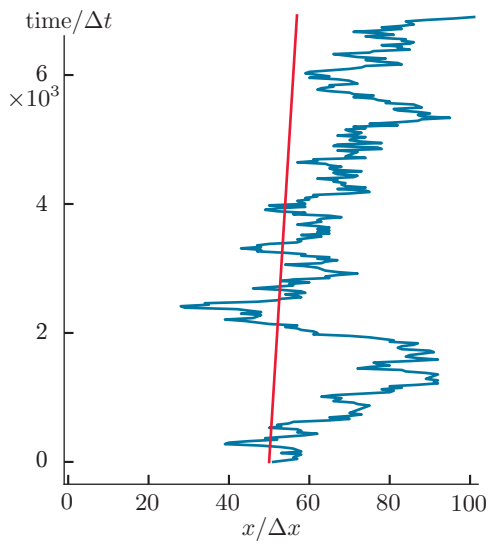

Figure 6.1: [Computer simulation.] **Typical random walk trajectory under constant applied force.** Time runs upward in this graph and is given as multiples of Δt. Position is given as multiples of Δx. A force of magnitude $0.002\zeta D/\Delta x$ is applied, directed to the right (increasing x). The resulting drift motion involves many temporary leftward excursions, but with an overall drift to the right. The average displacement over many instances has constant velocity given by Equation 6.2 (*red line*).

Your Turn 6A

a. Put together all the pieces to find that we can model Brownian motion with drift by choosing

$$\boxed{\mathcal{P}_+ = \tfrac{1}{2}\Big(1 - \frac{\Delta U}{2\zeta D}\Big).} \qquad \text{Physical model of Brownian motion on a landscape} \qquad (6.3)$$

Here ΔU refers to the difference in potential energy at two points separated by Δx, an approximation for $\Delta x(\mathrm{d}U/\mathrm{d}x)$.

b. Confirm that the expression just given is dimensionless, as it must be.

To appreciate Brownian motion with drift, Problem 6.2 asks you to simulate it. Figure 6.1 shows a typical motion. Over the short time scale shown, random excursions dominate, but over longer times the slow but relentless drift wins out over the violent but random kicks.[4]

6.3 RANDOM WALK IN A TRAP

6.3.1 A position-dependent stepping probability is a model for a force field

We are now ready to turn to molecular binding. Covalent chemical bonds are nearly permanent in life processes; they usually persist until broken by specialized machines (enzymes). However, most molecular recognition relies on weaker, more transient associations, for example, electrostatic interactions between charged groups, hydrophobic interactions, hydrogen bonds, and so on. These interactions are generally of short range, so that even if two molecules are matched and ready to bind, they remain

[4]It can be even more instructive to watch an animation of simulated data; see `DRIFTescapeTraj.mp4` in Media 6 or make your own (Problem 6.2).

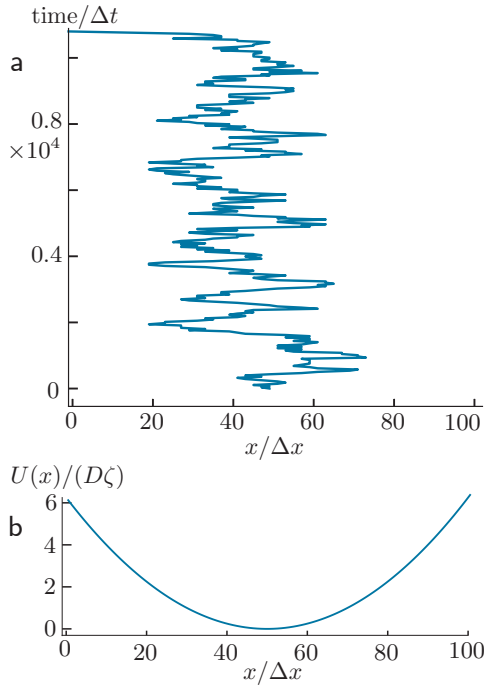

Figure 6.2: [Computer simulation.] **Random walk in a symmetric, harmonic potential energy trap.** (a) Although it is constantly pushed toward the center by a restoring force field, the walker eventually does arrive at $x = 0$. Media 6 displays this trajectory as an animation. (b) Potential energy trap giving rise to the walk in (a). The region most heavily visited by the walker aligns with the zone of low potential energy.

"unaware" of that fact until they accidentally blunder into one another. That last observation suggests that we could model binding by a random walk in a potential energy profile that remains "off" until two molecules are close to each other, and in nearly the proper orientation to bind.[5] Metaphorically the profile is often called a **landscape**; its maxima and minima are called "hills" and "valleys," and so on.

That is, the systematic force exerted on the walker *depends on its current position.* We can simulate that just as easily as we did drift, simply allowing for the possibility that the Bernoulli trial determining the outcome of each step has probability that depends on that step's starting point. Problem 6.3 will offer you some suggestions about how to handle this situation efficiently on a computer, but conceptually it should not be surprising. Our walker may blunder into a high-force region (large $|x|$ in Figure 6.2), but then it will be strongly pushed back toward its "home."

Figure 6.2a shows a typical trajectory in the potential energy trap described by

$$U(x) = 0.0025\zeta D\left(\tfrac{x}{\Delta x} - 50\right)^2. \tag{6.4}$$

This particular potential is famous in first-year physics, where we call it "the harmonic oscillator," but no periodic oscillation is visible in the figure—just noise. Nevertheless, there *is* a simple behavior hidden in this motion. We can find it by thinking probabilistically.

[5] T2 A more sophisticated treatment would replace potential energy by *free* energy throughout the following discussion.

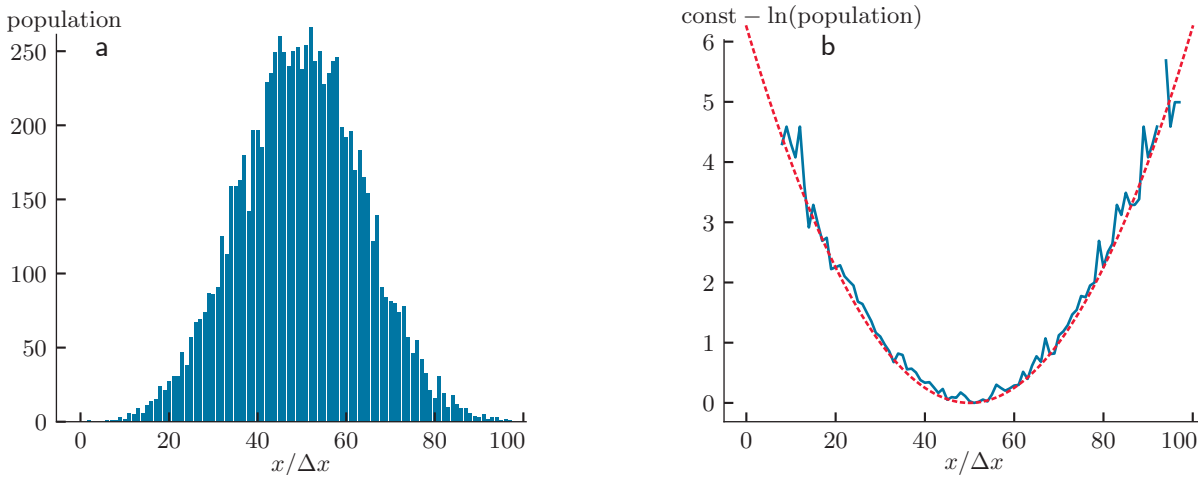

Figure 6.3: [Computer simulation.] **Distribution of 10 000 random walkers in a harmonic trap** after an initial equilibration time. (a) All walkers were released near the minimum of the trapping potential, but their distribution quickly approached the steady form shown here. (b) This semilog plot reveals the structure of the equilibrium distribution, by comparing it to $\exp(-U/(\zeta D))$ (*dotted red line*).

6.3.2 The Boltzmann distribution emerges after equilibration

Instead of looking at individual trajectories, let's think about them in aggregate. Regardless of where we release our walker, eventually it is likely to end up near its preferred location ($x = 50\Delta x$ in our example). Indeed, eventually it will *forget* its initial position. From then on, it will make a lot of small excursions about that point, as well as rarer big ones. The physically relevant question we may then ask is *what is the distribution of its positions* over many trials (or over a long time).

Figure 6.3a shows the answer to the last question, approximated with a finite number of walkers. Not surprisingly, they cluster near the center of the potential trap. What *may* be surprising is that this distribution is an old friend: Panel (b) shows that it is in fact a Gaussian.

Our result illustrates a far more general theme. The walker has arrived at a state called **thermal equilibrium**; books on statistical physics show that in equilibrium, the relative populations of various states are always given by:

$$\mathcal{P}(x) \propto \mathrm{e}^{-U(x)/k_\mathrm{B}T}. \qquad \textbf{Boltzmann distribution} \tag{6.5}$$

In this formula, T is absolute temperature and k_B denotes a constant of Nature (the **Boltzmann constant**) equal to $1.38{\cdot}10^{-23}\,\mathrm{J\,K^{-1}}$. Substituting our example Equation 6.4 into Equation 6.5 yields the Gaussian distribution that we indeed found.

Albert Einstein pointed out a remarkable aspect of the preceding argument. Our physical model involved two parameters that we made no attempt to calculate: the friction constant of the particle, ζ, and its diffusion constant, D. Each depends in a complicated way on the particle's size and shape, the temperature-dependent viscosity of the surrounding medium, and so on. Our simulation involved their product, which entered via Equation 6.3. Comparing our result to the Boltzmann distribution (Figure 6.3b) shows that

$$\zeta D = k_\mathrm{B}T. \qquad \textbf{Einstein relation} \tag{6.6}$$

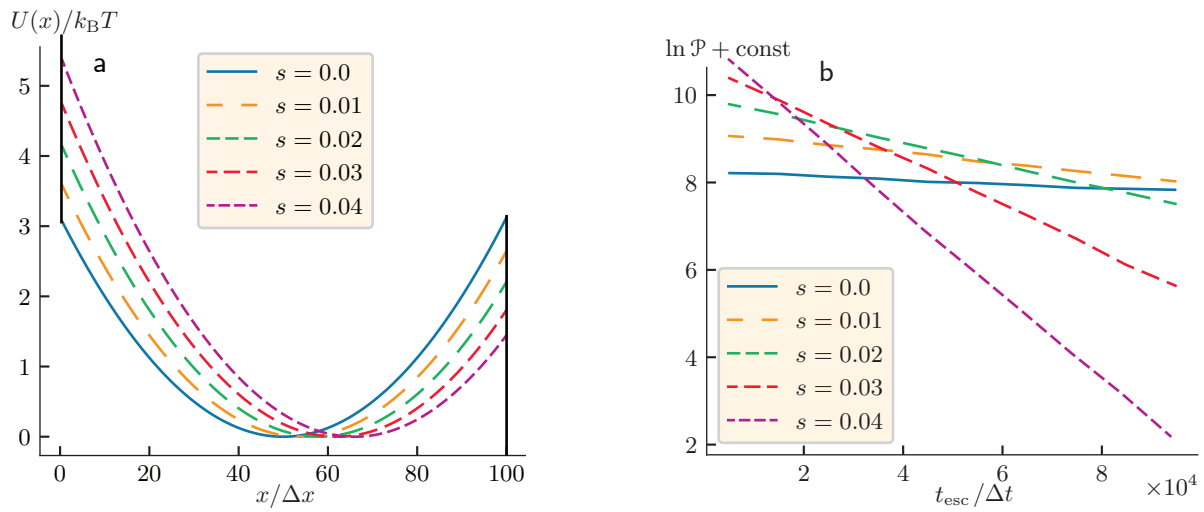

Figure 6.4: [Computer simulation.] **First-passage times for escape.** (a) Five different harmonic potentials. Each has a "hard wall" at $x = 0$ (*left vertical black line*). Each has a "cliff" at $x = 100\Delta x$ (*right vertical black line*) allowing "escape." The force parameter s is defined in Equation 6.7. (b) Semilog plot of the distributions of first-passage times. For each s value shown, 80 000 walkers were released near the center of the trap. Initially enough time was allowed to pass for the distribution to reach quasiequilibrium. Then a "clock" was started, and the times to escape after that moment were recorded. The figure shows a histogram of those times.

Equation 6.6 is actually universal; it holds for any potential energy trap, in any number of dimensions. It says that two complicated parameters describing nonequilibrium processes (friction and diffusive spreading) must always obey a *simple* relation dictated by *equilibrium* physics.

6.4 ESCAPE OVER A BARRIER

6.4.1 First passage time gives a quantitative, single-molecule replacement for the notion of rate

Section 6.3 characterized a physically bound state via Brownian motion in a potential energy trap. The position variable x represents the deformation of a bound complex. Let's now think about *un*binding. In everyday life, we are accustomed to systems like stuck doors or computer keyboards that do not respond to small forces, but do respond promptly and reliably if we pull or push "hard enough." The nanoworld behaves differently from this. If two bound particles are being pulled apart, they may jiggle for a long time before "escaping." And if we repeat the experiment many times, the waiting time for unbinding turns out to be a *random* variable. So instead of asking "How hard must I pull?" the relevant question is

> *What is the distribution of unbinding times, and how does it depend on applied force?*

To make progress, recall that physical binding forces are generally of short range. So although they may be created by a restoring force that increases with deformation, at some point they must "let go." We can model such behavior by a potential energy landscape with a "cliff," that is, one that drops suddenly to zero after some threshold

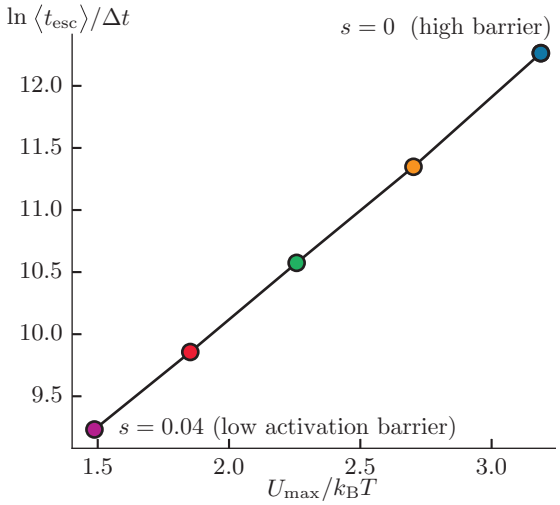

Figure 6.5: [Computer simulation.] **Mean first passage time versus energy barrier.** This semilog plot illustrates the general rule that, for a simple 1-step escape problem, mean first passage time is simply a constant times $\exp(U_{\rm max}/k_{\rm B}T)$. Values were calculated from the slopes of the lines in Figure 6.4b (or alternatively, via the procedure in Section 6.4′ on page 146).

value of x. Figure 6.4a shows several examples. Each is harmonic (a quadratic function) for small deformations:

$$U(x) = \zeta D\Big(0.0025\big(\tfrac{x}{\Delta x} - 50\big)^2 - 2s\frac{x}{\Delta x}\Big) + \text{const.} \tag{6.7}$$

Compared to the preceding example, each of these landscapes has a constant force controlled by a new parameter s. Each also has a "hard wall" that forbids deformation below $x = 0$. But each has a "cliff" that lets the walker escape permanently to a region of very low potential energy if it ever arrives at $x = 101\Delta x$. Such a random walker will always unbind eventually, so its final (equilibrium) distribution is not very interesting. But it may take quite a long time to arrive at that final state. Each of the five examples shown has a different "cliff" height $U_{\rm max}$, called the **activation barrier** to escape. To escape, the walker must gain enough energy from the constant thermal kicks to surmount this barrier.

We would like to know about the probability distribution of the **first passage time**, that is, the moment when the walker irrevocably falls off the cliff. If the typical wait is long compared to the equilibration time, then the particle will initially wander in a **quasiequilibrium** state resembling true equilibrium in a trap with no exit. Figure 6.4b shows results from a situation of this sort. Not surprisingly, the walkers with lower activation barrier escaped faster on average (their PDF places more emphasis on smaller values of $t_{\rm esc}$). Once again, however, we find greater simplicity than we might have expected:

- *The distribution of escape times in our discrete-time simulation is always Geometric.*[6]
- *The mean first passage time is a constant times the exponential of the activation barrier.*

(6.8)

To establish the first of these results, note that Equation 3.13 on page 45 gives

$$\log_{10} \mathcal{P}_{\rm geom}(j) = \text{const.} + j \log_{10}(1 - \xi),$$

[6]Section 3.4.4 on page 44 introduced this family of discrete distributions.

where $j = t/\Delta t$ is the attempt number of the first "success" (exit or unbinding) and ξ is the one parameter describing a Geometric distribution. This expression is linear in j, which matches the behavior seen in Figure 6.4b.

To establish the second result in Idea 6.8, first note that because ξ is small, we have $\ln(1-\xi) \approx -\xi$, and a similar result for the common log.[7] So we can get ξ by finding the *slope* of the semilog plot in Figure 6.4b and dividing by $-\ln 10$. You found in Problem 4.22 that the expectation of j is ξ^{-1}; Figure 6.5 plots this quantity, and shows that, as claimed, it indeed varies linearly with activation barrier.[8]

Chapter 9 will show that the mean rate of escape is $(\langle j \rangle \Delta t)^{-1}$, so we see that this rate is proportional to $\exp(-U_{\max}/k_{\mathrm{B}}T)$, a famous rule of thumb for chemical reactions often called the **Arrhenius rule**.

6.4.2 In simple situations, pulling speeds up unbinding

As with our study of drift, it can be very enlightening to watch an animation of typical trajectories.[9] It becomes clear that:

- The walker is not "trying to get out." It doesn't even "know" that there *is* a way out.
- The walker is not "creeping up toward the exit." It's just blundering around, and eventually it stumbles upon the exit. Meanwhile it often "wastes" lots of time on excursions in the "wrong" direction.

A second kind of animation is also useful, showing the evolution of the complete probability distribution of a large number of trials all starting at the minimum of the potential.[10] We see that:

- After an equilibration time, the probability distribution approaches a form that is independent of the initial distribution.
- Probability then "leaks out" slowly over the cliff, because the region just inside the cliff is so rarely visited.

The second of these points also explains Idea 6.8: The probability to escape depends on the fraction of walkers poised to escape, and in a quasiequilibrium situation, that fraction is approximately governed by the Boltzmann distribution.

Although our physical model is primitive, it at least incorporates the notion of an external pulling force: Each of the energy profiles in Figure 6.4a is related to the blue one by adding a *linear* term to the potential energy—and a linear term corresponds to a constant force.[11] We may take one of the curves to be the "intrinsic" energy profile of binding, and the various linear additions to represent "external" forces. We then see that, unsurprisingly,

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[7] See page 17.

[8] T2 Our result does not depend on the initial position of the walker in its trap, because we let it wander for at least the equilibration time before starting the "clock," erasing any memory of its initial position.

[9] See `escapeTraj.mp4` in Media 6 or make your own (Problem 6.5). Also Media 3 shows two real mesoscopic examples: a micrometer-size bead moving in a potential created by a DNA tether, and another in a double trap created by optical tweezers.

[10] See `escapeHisto01-side.mp4` in Media 6, or make your own (Problem 6.5).

[11] In addition, a constant has been added to each curve to make its minimal value equal zero. Adding a constant to the potential energy makes no change in the corresponding force.

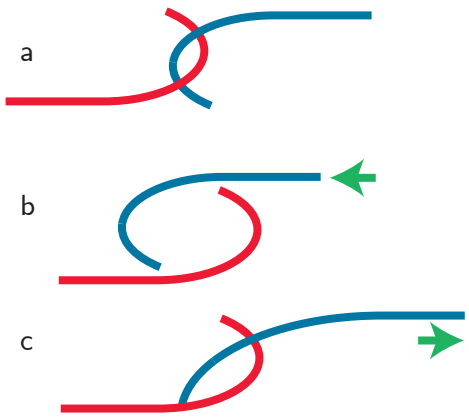

Figure 6.6: [Metaphor.] **Mechanical model of a catch bond.** (a) Two deformable hooks are linked. A weak spring keeps them under slight tension (*not shown*). (b) However, thermal agitation can move them together, against the weak spring, far enough to disengage. (c) An external pulling force can discourage that escape pathway. Then the hooks stay engaged unless the external force is so large as to straighten one or both of them (the alternate pathway to escape).

- Pulling toward the right adds $-sk_{\mathrm{B}}T(x/\Delta x)$ to the potential energy, where s is a positive constant describing how hard we pull.
- A positive value of s draws down the barrier to escape, ...
- which in turn speeds up unbinding.

What we have gained over those qualitative comments is a quantitative understanding that can make predictions about other force values not yet tested (Figure 6.4b). This unsurprising behavior is generically called **slip bonding**.

T2 *Section 6.4′ on page 146 works out how Figure 6.5 was derived from data in Figure 6.4, and hints at a more general treatment of escape.*

6.5 BONDS. CATCH BONDS.

6.5.1 More complex molecular pairs can have multiple unbinding pathways

At last we can return to this chapter's seemingly paradoxical Focus Question. Can a bond become "stronger" when we try to pull it apart? Section 6.4 explained how to make such questions more precise. We really wish to ask, "Can the mean lifetime of a bond increase when it is under load, compared to when it is not?" If so, we'll say our system exhibits **catch bond** behavior, in contrast to slip bonding.

Figure 6.6 illustrates how catch bonding might arise mechanically. Think of the hook systems that many plants (such as burdock) use to hitchhike their seeds on the fur of passing animals. The third panel of the figure depicts a rightward pull that discourages the easy escape route (middle panel), leading to catch bonding. However, high enough force can overcome the barrier for the hard escape route, and the system reverts to slip bonding. We now ask how this scenario works in the microworld.

Figure 6.7a shows several energy landscapes that each provide *two* pathways to unbinding: one to the left and another to the right. We can simulate that system just as easily as the preceding ones, terminating each walk if the walker ever crosses *either* $x = 0$ or $x = 100$. Panel (b) shows that once again, the probability distribution of escape times is Geometric for every applied force. We also see a reassuring symmetry in the results: Compared to the purple profile, pulling to the left speeds up escape to the left (blue curve), whereas pulling equally strongly to the right speeds up escape to the right by the same amount (the olive curve superimposes on the blue dashed one

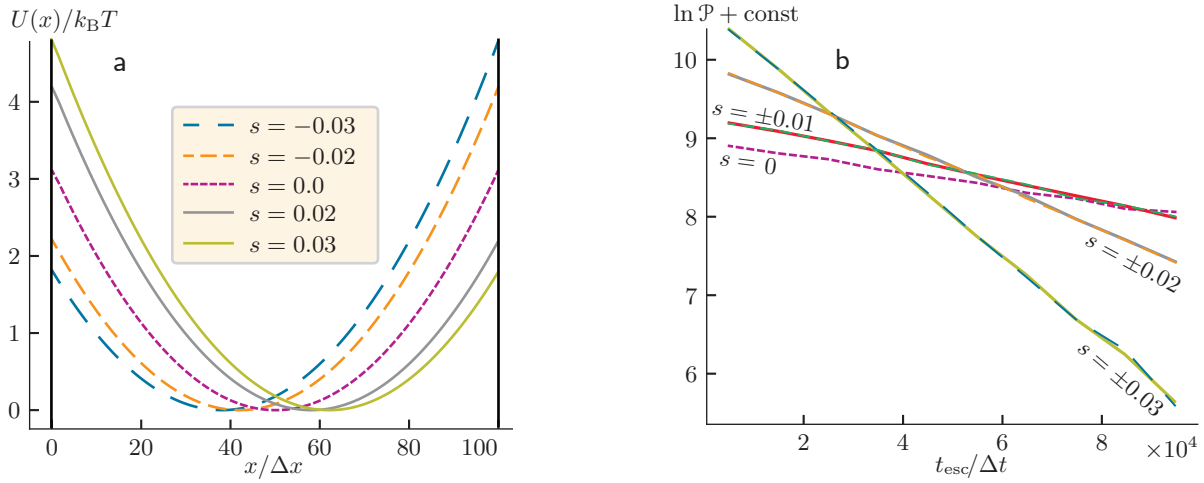

Figure 6.7: [Computer simulation.] **Multiple escape routes.** 80 000 walkers were again released, but this time they could "escape" either to the left or right side of the harmonic trap. (a) Potential energy functions. Negative values of s correspond to easier escape to the left; positive values correspond to easier escape to the right. (b) Semilog plot of the distribution of first-passage times.

in panel (b)).

But suppose that the two escape routes are intrinsically different. For example, the unstressed bond may be described by one easy and one hard exit route (orange curve in the figure). In that case, pulling to the right slows exit to the left (purple curve). It also speeds up exit to the right, but *only the fastest exit route matters,* and that one is getting slowed. This in a nutshell is the catch-bonding phenomenon.

The point is worth repeating in different words: For the purple curve, the two exit routes (right and left) are equally fast because they have equal activation barriers. But both have higher activation barrier than the left exit in the orange curve, so the unbinding time increases if we shift the system from orange to purple by pulling on the bond.

Certainly if we pull hard enough, then eventually exit to the *right* becomes the dominant (fastest) mode (gray curve), and pulling harder still (olive curve) will make that exit faster than at zero force. That is, a catch bond will under sufficient force revert to more intuitive ("slip bond") behavior. But at intermediate forces,

> *Mean first passage time can increase with increasing applied force in a catch-bond arrangement.*

Figure 6.8 bears out the qualitative expectations from our mechanical metaphor. Each colored dot corresponds to one of the systems in Figure 6.7. The left part of the graph shows that the usual relation between escape time and activation barrier holds as long as the right-side barrier is the controlling (lowest) one. That is the familiar slip-bonding regime. When that is not the case, however, the right side of the graph shows that bond lifetime can rise despite an increase in applied force.

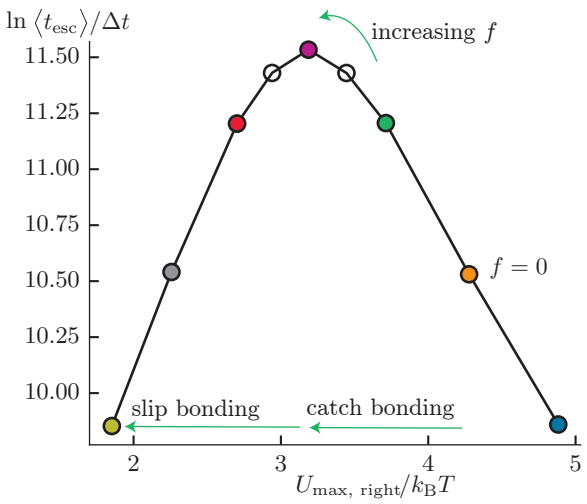

Figure 6.8: [Computer simulation.] **Mean bond lifetime is controlled by the lowest energy barrier.** See text. The colors correspond to those in Figure 6.7. We imagine a system in which the orange dot corresponds to zero external force. Imposing an external force directed to the right then lowers the activation barrier $U_{max,\,right}$ for escape to the right, and hence moves to the left on this graph. Small external forces increase bond lifetime (catch bonding, *green and purple dots*), whereas at larger forces lifetime decreases (slip bonding, *red, gray, and olive dots*).

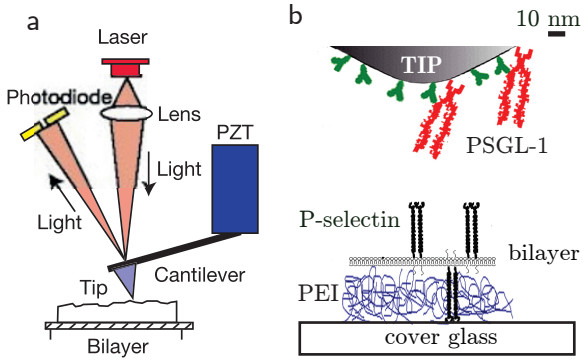

Figure 6.9: [Schematics.] **An experiment to study catch bonding at the single molecule level.** (a) The atomic force microscope (AFM) attaches an atomically-sharp probe tip to a flexible arm (labeled *cantilever*). A piezoelectric transducer (*PZT*) then brings it down to touch a surface. Observation of the tip's motion lets the experimenter infer both the distance between tip and bilayer, and the force that they exert on each other. (b) Closeup. In this experiment, the surface was covered with a gel layer (*PEI*), then an artificial bilayer with embedded adhesion molecules of interest (*P-selectin*). The AFM tip is decorated with peptidoglycans (*PSGL-1*), so that the junction mimics that between a leukocyte and the wall of a blood vessel. [From Marshall et al., 2003.]

6.5.2 Single-molecule experiments yield the entire distribution of unbinding times

Certainly real molecular recognition is more complicated than Figure 6.6! But our main goal was just to answer, "How could anything like catch bonding possibly happen at all?"

Many scenarios of this sort have been established by examining the structure of molecular binding partners. For example, a recognition molecule can, under tension, deform to reveal a second binding site, which then engages a second domain on the ligand molecule, strengthening their grip.

Figure 6.9 sketches an early experimental test of catch bond formation at the level of single molecules: A tiny mechanical actuator brought two surfaces into contact, then monitored their separation while applying precisely controlled pulling forces. Figure 6.10a shows bond lifetime distributions similar to those from our model

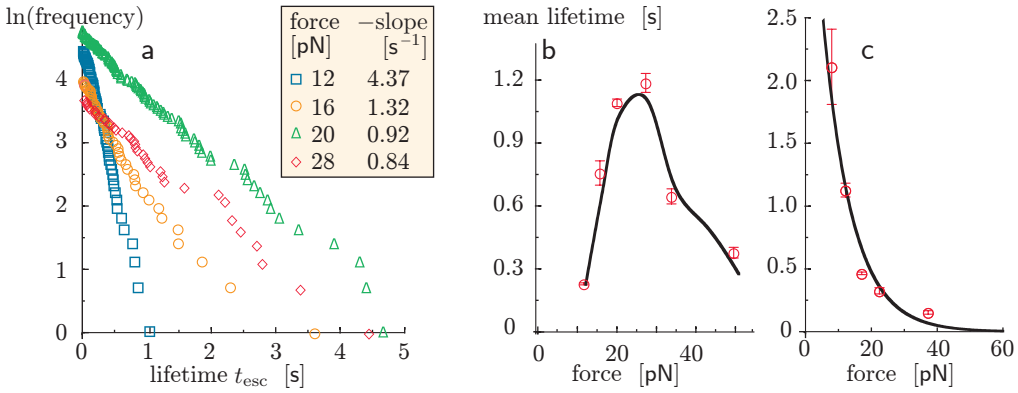

Figure 6.10: [Experimental data.] **Bond lifetimes in the experiment of Figure 6.9.** (a) Semilog plot of the number of events with a lifetime of t_{esc} or more versus t_{esc} for PSGL-1 binding to P-selectin. Various constant pulling forces in the catch bond regime were applied. Compare the simulation results (Figures 6.4b and 6.7b). (b) Mean lifetimes $\langle t_{esc} \rangle$ estimated as -1/slope of the plots in (a). Compare the simulation results in Figure 6.8. (c) For comparison, a similar plot but PSGL-1 was replaced by an antibody; ordinary slip-bond behavior was observed. [Data from Marshall et al., 2003.]

(Figure 6.7b), including the hallmark of catch bonding: A peak in mean bond lifetime at nonzero pulling force.

6.6 BIOPHYSICAL IMPLICATIONS

6.6.1 Immune cell activation involves catch bonding

Why would a cell find catch bonding useful? Every cell in your body constantly advertises its contents by chopping up old or damaged proteins, transporting the fragments (peptides) to its surface, exporting them, and displaying them on "billboards" called **major histocompatibility complexes** (MHCs; see Figure 6.11). Also specialized cells, called **antigen presenting cells**, engulf and digest free viruses and bacteria, and present fragments of their proteins on similar billboards.

Our immune system includes migratory cells that constantly move through our bodies, encountering our other cells and interrogating their health by examining the peptides displayed on their MHCs. A cell that is cancerous, infected by a virus, or otherwise irremediably in trouble will display unusual peptides; immune cells such as **T-lymphocytes** (T cells) can recognize such cells, engaging a chain of events that results in killing them before they can proliferate (in the case of cancer) or generate new virions (in the case of viral infection). Each T cell is only looking for a few particular peptides, but there are a lot of T cells, with a diverse repertoire of potential targets.

T cells must exercise exquisite judgement. Every cell displays tens of thousands of normal ("self") peptides; even a sick cell displays only a few abnormal ("non-self") ones. So even a tiny false-positive recognition rate would cause the immune system to attack our cells indiscriminately. (Indeed, autoimmune disorders do involve such errors, but they are rare.) How can immune recognition be so very accurate?

Part of the answer is now unfolding. Immune cells bristle with receptor molecules that recognize non-self peptides when they are displayed by another cell's peptide–MHCs. Upon cell-cell contact, those receptors find and bind their partners, if any. The

Figure 6.11: [Artist's reconstructions based on structural data.] **T cell activation.** A key moment in the dialog between cells of the immune system, when an antigen presenting cell (*top*) is displaying a protein fragment (peptide, *red dot at center*) with MHC, and uses it to trigger activation of a T cell (*bottom*) through T-cell receptors. [Art by David S Goodsell from coordinates in the RCSB Protein Data Bank: `doi:10.2210/rcsb_pdb/goodsell-gallery-022`.]

T cell then monitors the *time* spent in the bound state, and only becomes activated if that time exceeds a threshold (typically several seconds). For even greater specificity, the T cell actively *tries to pull apart* the receptor-peptide complex, for example, by its normal crawling motion when it comes to any surface. The resulting mechanical force can lead to catch bonding if the receptor has found its matching peptide, leading to an extended bond lifetime and greater chance of meeting the threshold time for T cell activation.

Several research groups have now documented parts of the preceding scenario. Some experiments are performed in vitro, with receptors extracted from T cells and a peptide–MHC complex known to activate that particular T cell class (Figure 6.12). Other experiments, involving individual living T cells, showed that indeed, sustained pulling force can enhance activation (Figure 6.13). Interestingly, the *direction* of the force was found to be significant: Force applied perpendicular to the cell membrane triggered very few cells, and even these responded minimally. In contrast, a tangential

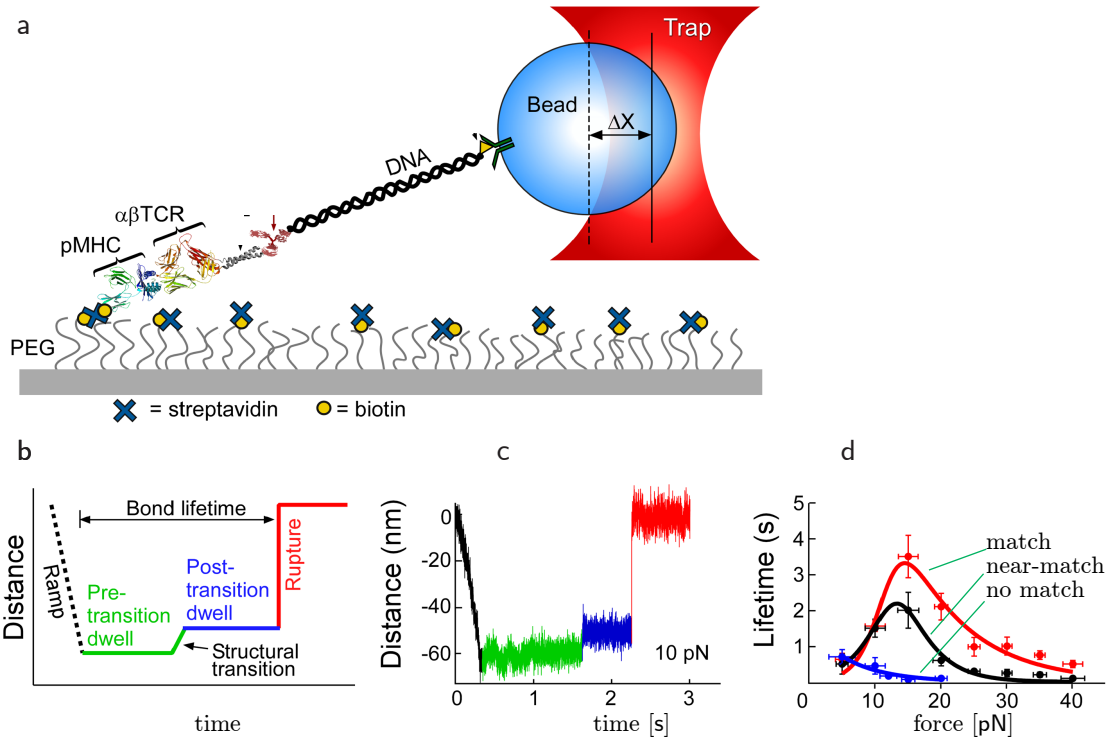

Figure 6.12: [Schematics; experimental data.] **In vitro catch bond assay** investigating the binding between a T cell receptor (indicated by $\alpha\beta TCR$) and the peptide-major histocompatibility complex that it recognizes ($pMHC$). (a) An optical tweezers instrument applies force to a micrometer scale bead (*not drawn to scale*), which in turn pulls on the T cell receptor via a tether made of DNA. A feedback system maintains constant pulling force by adjusting the location of the trap so that the bead is slightly off center in its potential energy landscape, and hence is pulled with a known force toward the center. The resulting force is transmitted by the tether to the binding pair. (b) Terminology. An initial "ramp" phase (*black dashed line*), loads the tether to a fixed force. Wild-type molecules usually underwent a structural transition before separating altogether. (c) Typical time series of bead position for applied force 10 pN, showing a dwell, transition, additional dwell, and final bond breakdown (rupture). (d) Force-lifetime plots for wild-type receptor presented with various peptide–MHCs. The *red* curve shows binding to the preferred peptide, for which this receptor is specific. The *black* curve shows binding to a modified peptide, differing from the preferred one by a single amino acid. The *blue* curve shows binding to a non-agonist peptide. Although the binding lifetimes for preferred and modified peptides were similar at the lowest applied force, they differed by nearly a factor of two in the optimally loaded case. [From Das et al., 2015.]

force of equal magnitude triggered a large fraction of cells, which responded vigorously (Figure 6.13b–d). Significantly, tangential forces are generated when a T cell crawls over another cell. The experiments also demonstrated that only a few binding pairs of molecules need to be engaged in order to trigger T cell activation. In short,

> *A T cell responds weakly, even if the correct peptide–MHC is engaged, unless a mechanical force is also applied. But with the appropriate mechanical force—even if applied by a nonliving apparatus—the same T cell can reliably activate even if* ***just two*** *of its receptors are engaged, while ignoring tens of thousands of similar but inappropriate peptide–MHCs.*

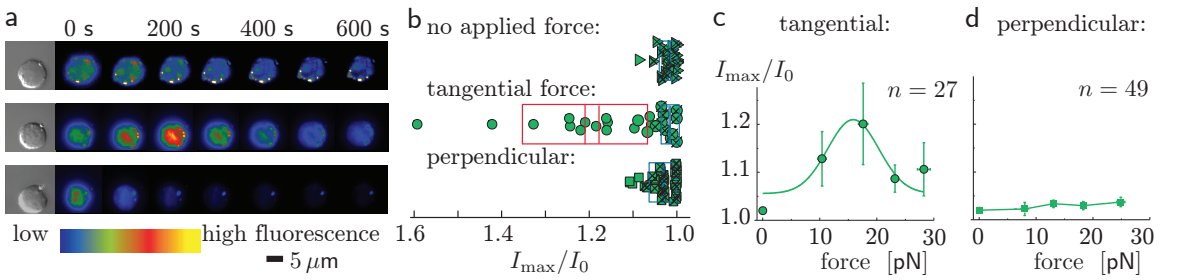

Figure 6.13: [Microscopy images; experimental data.] **Triggering of T cells by pMHCs.** An optical tweezer setup was constructed, similar to that in Figure 6.12 but without the long DNA tether and hence able to present a living T cell with multiple copies of a chosen peptide–MHC, bound directly to the probe bead. In the data shown here, the surface density of pMHC on the bead gave rise to about 29 contacts when the bead was brought into contact with the T cell; also the peptide chosen was the one that best matched the T cell's receptors. (a) In each row of colored images, a calcium-sensitive fluorescent dye was used to visualize the T cell's response to antigen presentation over time. The probe bead is visible as a dot in the 1 o'clock position. Each row corresponds to a force regime described in (b); the middle set of conditions gave sustained cell activation. (b) Quantitative comparison of peak calcium signal I_{max} for each trial (*symbols*), between trials with no applied force, and with force between 10 and 25 pN directed tangentially to the cell membrane, or directed perpendicular to the membrane. Also each single cell's response was classified as triggered (activated) or not based on its entire time course of fluorescence: Untriggered cells are shown as *crossed* symbols and summarized by *blue* box plots. Triggered cells are shown as *open* symbols and summarized by *red* box plots. Binding without subsequent application of force rarely led to cell triggering (*top*). (c) Averaged responses over many trials for force applied parallel to the cell membrane. Comparison of various applied forces shows catch bond behavior. (d) For this choice of antigen density, no significant triggering occurred at any perpendicular force. [From Feng et al., 2017.]

6.6.2 Leukocyte rolling also relies on catch bonds

An activated T cell can do more than just destroy the cell that activated it: Once it has detected trouble, activation also switches cell division into high gear, creating a large population of T cells all with the same specific receptors, ready to hunt down additional sick cells. First, however, the T cells must find their targets.

Actually, T cells are just one of several classes of white blood cells (collectively called **leukocytes**). Many circulate in the blood vessels, sniffing for chemical markers of inflammation (**cytokines**) laid down by another part of the immune system. When a leukocyte encounters raised cytokine levels, it attaches to the inner wall of a blood vessel (the **endothelium**), penetrates it, and begins crawling through the surrounding tissue.[12] How do they do this?

In greater detail, a leukocyte adheres to the endothelium via transient bonds that let it *roll* along the blood vessel, scanning the surface for cytokines. When it finds what it's looking for, it binds more tightly and begins the process of exiting the blood vessel, but already the initial rolling state is of great interest. How does the leukocyte know not to adhere to other blood cells? How does it know to adhere in larger vessels, but not in the capillaries?

Catch bond formation is now understood as one key to answering the preceding questions. For example, a class of molecules called glycoproteins bind most strongly to partners (selectins) protruding from endothelial cells, with strongest binding at pulling force of around 20 pN (Figures 6.9–6.10). A leukocyte floating freely along in

[12] Less benignly, metastatic cancer cells also circulate in the blood and can similarly exit to found new colonies far from the original tumor.

the middle of the blood vessel may encounter other cells and briefly adhere, but they, too, are borne along by the same flow and there is little net force between the two cells, leading to little adhesion. But when the leukocyte adheres to a stationary endothelial cell, its catch bonds are stretched, and become longer lived, giving rise to the rolling phenomenon. Moreover, blood vessels with stronger flow lead to longer-lived catch bonds, a compensation mechanism that ensures that the leukocytes maintain the optimal rolling velocity over a range of different flow rates. In capillaries, with the slowest flow rate, there is little catch bonding.

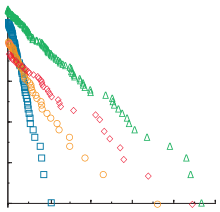

Fig. 6.10a, p. 140

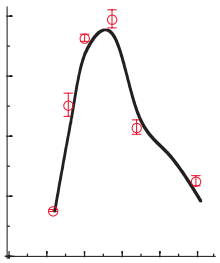

Fig. 6.10, p. 140

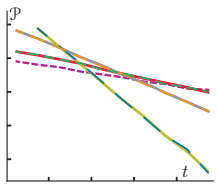

Fig. 6.7b, p. 138

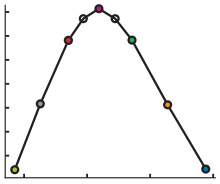

Fig. 6.8, p. 139

THE BIG PICTURE

Our physical model (Idea 6.3) was absurdly simple, but it nevertheless contained a lot of buried treasure: The basic facts about free Brownian motion, drift under constant force, equilibration in a trapping potential field, the Boltzmann distribution in equilibrium, the Arrhenius rule for escape in quasiequilibrium, and the entire surprising phenomenon of catch bonding. The key step was to understand bond breaking as a *first passage* problem. Although evolved living systems are more elaborate than our model, still data such as those in Figures 6.10a,b do look strikingly like our simulation results (Figures 6.7b and 6.8).

KEY FORMULAS

- *Diffusion:* For steps $\pm\Delta x$ in one dimension, every time interval Δt, the diffusion constant is
$$D = \Delta x^2/(2\Delta t). \qquad \text{[6.1, page 130]}$$
- *Drift:* In the nanoworld pulling a particle with force f superimposes a drift velocity
$$\langle v_{\text{drift}} \rangle = f/\zeta \qquad \text{[6.2, page 130]}$$
on its usual Brownian motion. The friction constant ζ is sometimes expressed in terms of its reciprocal, the mobility.
- *Landscape:* The motion of a particle in a potential energy profile $U(x)$ can be modeled by a random walk with position-dependent probability to step rightward:
$$\mathcal{P}_+ = \tfrac{1}{2}\Big(1 - \frac{\Delta U}{2\zeta D}\Big). \qquad \text{[6.3, page 131]}$$
- *Boltzmann distribution:* In thermal equilibrium, the relative populations of various states obey
$$\mathcal{P}(x) \propto \mathrm{e}^{-U(x)/k_{\mathrm{B}}T}. \qquad \text{[6.5, page 133]}$$
- *Einstein relation:*
$$\zeta D = k_{\mathrm{B}}T. \qquad \text{[6.6, page 133]}$$
- *Arrhenius rule:* The distribution of escape times in our discrete-time simulation is Geometric, with mean first passage time proportional to the exponential of activation barrier/$k_{\mathrm{B}}T$. In this expression T is absolute temperature and k_{B} is a constant of Nature.

FURTHER READING

Semipopular:
Mlodinow, 2008.

Intermediate:
Immune system: Sompayrac, 2019.

Technical:
Catch bonds: Thomas et al., 2008. Experimental discovery: Thomas et al., 2002; Marshall et al., 2003. In T cells: Liu et al., 2014; Das et al., 2015; Hu & Butte, 2016; Feng et al., 2017; James, 2017. In a molecular motor system: Nord et al., 2017. Studied via mean first-passage time: Vrusch & Storm, 2018.
Leukocyte rolling: Huse, 2017. More detailed look at the P-selectin bond: Evans et al., 2004.

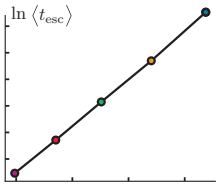

Fig. 6.5, p. 135

Track 2

6.4′a Mean first passage time from simulation data

Our simulation results suggested that, after equilibration, the distribution of escape times was Geometric. But to find the mean first passage times shown in Figure 6.5, we need to estimate the parameter ξ describing the Geometric distribution generated by each simulation.

We cannot run each simulated trajectory all the way out to time infinity, so in many instances, the simulation terminated before the walker had a chance to escape. Such instances were discarded, so Figure 6.4b on page 134 really shows only the distribution *truncated* to the first K steps. If we just computed the mean of those escape times, we would be looking at a biased sample, and hence underestimating the mean lifetime.

To do better, we could lay a ruler along the curves in Figure 6.4b and estimate their slopes. Or we could resort to advanced fitting ideas from Chapter 7 of this book. But there is a simpler alternative. Each time a simulation *does* end with escape, we log the number of time steps required, that is, the first-passage time j. Then we compute the average $\langle j \rangle_K$ of all the reported j values, with the understanding that $j < K$. We can then work out the relation between ξ, K, and $\langle j \rangle_K$ and use it to solve for ξ given the known K and the observed $\langle j \rangle_K$.

To find the required relation, we follow some familiar steps:

$$\langle j \rangle_K = \Big[\sum_{j=1}^{K} j\xi(1-\xi)^{j-1}\Big] \Big/ \Big[\sum_{j=1}^{K} \xi(1-\xi)^{j-1}\Big].$$

$$= \frac{-\xi \frac{\mathrm{d}}{\mathrm{d}\xi}\big((1-\xi)(1+\cdots+(1-\xi)^{K-1})\big)}{\xi\big(1+\cdots+(1-\xi)^{K-1}\big)}.$$

Your Turn 6B

Finish simplifying this expression to get the desired functional relation. [*Hint:* Make sure your result behaves reasonably in the limiting case where $\xi \to 0$ at fixed K, and also in the case $K \to \infty$ at fixed ξ.]

Finally, the expectation of a Geometric distribution[13] is ξ^{-1}. This is the quantity plotted in the main text.

6.4′b Kramers approach

The simulation approach taken in the main text is simple, direct, and concrete. But such approaches may leave us wondering how general our results are. H. Kramers developed a more general, analytic approach to thermal escape problems in 1940. His derivation involved writing a master equation for the probability distribution of positions,[14] and confirmed that quite generally, if a single reaction coordinate with a single dominant barrier can be used to describe unbinding, then the mean first passage time obeys the Arrhenius rule (Equation 6.8 on page 135). Kramers also gave a useful approximate formula for the prefactor multiplying the exponential.

[13] See Problem 4.22.
[14] See Section 10.3.4′ on page 255.

Track 2

6.6.1′ More about T cell activation

Careful experiments were done to control the binding duration of a ligand to a T cell, leaving every other condition unchanged. These experiments confirmed the statement in the text that this duration is critical for triggering the receptors and then activating the T cell (Yousefi et al., 2019; Tischer & Weiner, 2014; Tischer & Weiner, 2019). Actually, however, multiple short binding events in rapid temporal sequence and in close spatial proximity can also trigger T cell receptors (Lin et al., 2019).

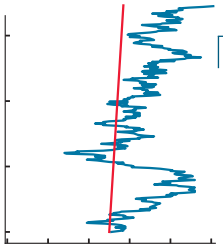

Fig. 6.1, p. 131

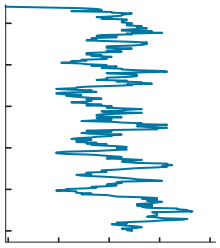

Fig. 6.2a, p. 132

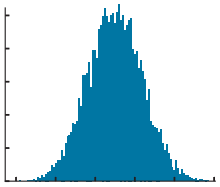

Fig. 6.3a, p. 133

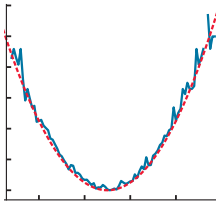

Fig. 6.3b, p. 133

PROBLEMS

6.1 *Finger trap*
[Not ready yet.]

6.2 *Diffusive motion with drift*
Write a computer code to generate data like Figure 6.1.

a. Specifically, substitute the constant force described in the figure's caption into Equation 6.3 to define a position-dependent Bernoulli trial distribution. Then generate a sequence of x values that all start at the center point, $x = 50\Delta x$, and that proceed for 7000 steps. Try making many such sequences and displaying the first few. Then at each step, find the average of all your instances at that step to clarify the average drift.

b. T2 Make an animated graphic of a representative trajectory.

6.3 *Motion in a trap*
Write a computer code to generate data like Figure 6.2a.

a. Specifically, use the potential energy function in Equation 6.4. But forbid the particle from leaving the range $0 \le x \le 100\Delta x$, as follows: If $x = 0$, set $\mathcal{P}_- = 0$, and if $x = 99$, set $\mathcal{P}_+ = 0$. Physically, we can imagine "hard walls" ($U = \infty$) at these locations. As in the preceding problem, release every walker from the center position.

b. T2 Make an animated graphic of a representative trajectory.

6.4 *Equilibrium distribution in a trap*
Write a computer code to generate data like Figure 6.3a,b. Specifically, model 10 000 trajectories, each with 50 000 steps and each starting from the center position. As in the preceding problem, use the potential energy function in Equation 6.4 and implement hard walls at the ends. It may start to get computationally intensive to simulate half a billion steps! But here is a time-saving trick.

For this problem, we don't attempt to follow the individual trajectories. All we need are the *populations* at each spatial position, for each time. Thus, your code need only retain an array of those populations. Also, the problem has the Markov property that each walker's next step depends only on its current position, not on its past history. So you can proceed as follows:

- For each time step, first make a new array to hold the populations at the next time step.
- Then consider each location ($k = x/\Delta x = 0, \ldots, 99$) in turn. If the population at position k is not zero, it will get partitioned into a subpopulation stepping right, with probability $\mathcal{P}_+(k)$, and those stepping left, with probability $1 - \mathcal{P}_+$. The partitioning is random, but Section 4.2.2 on page 68 argued that it follows a Binomial distribution. Thus, a *single* draw from the appropriate Binomial will establish the fates of *all* walkers currently at k.[15]
- Use the preceding result to update the new populations at $k - 1$ and $k + 1$, respectively. Step through all k values.

[15] You'll need a special rule at $k = 0$: All walkers step right due to the hard wall, and similarly at $k = 99$.

- Copy the updated populations into the main population counter array and repeat for the desired number of time steps.

a. Carry out the above steps and show the final distribution.

b. If you released all the walkers exactly at $x = 50\Delta x$, then your graph will have an unpleasant jagged character. Why did that happen? Try releasing just half of the walkers at 50 and the the other half at 49. Why does that help?

c. T2 Make an animated graphic of the time evolution of the probability distribution, estimated from your finite sample. How quickly does it reach nearly equilibrium form? How much does it then jitter around that form?

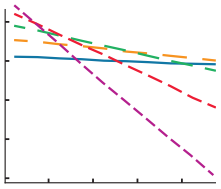

Fig. 6.4b, p. 134

6.5 T2 *Probability leakage*

Return to the preceding problem, but this time relax the hard wall on one side to generate graphs like Figure 6.4b, as follows. Instead of forbidding a walker at $x/\Delta x = 99$ from stepping right, we allow it with the usual formula for $\mathcal{P}_+$, but then permanently remove it from the population of steppers: It has "escaped."

a. Choose an interesting value of the parameter s appearing in Equation 6.7 on page 135. Run your simulation long enough to get a good sample of escapes, record the times when each escapee made its last step, and find the distribution of those times.

b. T2 Make an animated graphic of the time evolution of the probability distribution, including one extra bin to represent the escapees, and describe what you see.

6.6 T2 *Isomerization modeled by a double trap*

Many macromolecules have multiple conformations that are each local minima of their (free) energy function. In this problem you'll model such situations and look at spontaneous (thermally induced) transitions between metastable states (**isomerization**).

a. Make a graph of the potential energy function

$$\frac{U}{k_B T} = 12\Big(\frac{1}{4}\big(\frac{x - x_*}{40\Delta x}\big)^4 - \frac{1}{2}\big(\frac{x - x_*}{40\Delta x}\big)^2\Big),$$

where $0 \le x \le 100\Delta x$ and $x_* = 50\Delta x$. Use this energy landscape to set up a random walk like those in previous problems, with hard walls at each end.

b. Release many trajectories all starting at the left-hand minimum, $x = 10\Delta x$. Let each one evolve for 7000 time steps. If any trajectories ever cross x_*, show a representative example.

c. Show the estimated probability distribution of positions at $t = 7000\Delta t$ and comment.

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